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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: J. Clark & C. Denning

Filing Date: June 13, 2000

Serial No: 09/593,316

Docket: 730/002

Title: ANIMAL TISSUE FOR XENOTRANSPLANTATION

Art Unit: 1632

Examiner: Quan J. Li, Ph.D.

APPEAL BRIEF

Commissioner for Patents and Trademarks
Alexandria, VA 22313

Dear Sir,

Applicant hereby appeals from the final Office Action dated January 30, 2003, rejecting all claims under examination in this application. This paper is appellant's Brief, pursuant to 37 CFR § 1.192.

A Notice of Appeal was filed in this application on June 4, 2003, setting the deadline for filing an Appeal Brief to August 4, 2003. This Brief is accompanied by a request for a one-month extension of time, along with authorization to charge the Deposit Account with the requisite fees.

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Applicant is grateful to Examiner Quan J. Li for her careful consideration of this application and applicant's arguments relating to the patentability of the claimed invention. Applicant is also grateful to the Examiner and her supervisor for meeting with applicant's representative on several occasions in an attempt to resolve the unresolved issues.

Nevertheless, the claims under examination still stand rejected, making this Appeal necessary to preserve applicant's intellectual property rights in this invention.

The Board of Patent Appeals and Interferences is respectfully requested to reverse rejection of the claims under examination, in view of the following remarks.

STATEMENTS PURSUANT TO 37 CFR § 1.192

Real Party in Interest

The real party in interest for the claimed invention is Geron Corporation, a Delaware corporation, to which the application and the claimed invention has been assigned in their entirety.

Related Appeals and Interferences

No other appeals or interferences are known by applicant or its representative that would directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

Status of claims

This application was filed with claims 1-32, which were the subject of a restriction requirement on September 25, 2001. Claims 1-6 were elected for prosecution on the merits, and claims 13-15 were rejoined into the group under examination on November 23, 2001 (Paper No. 7). Claims 8-12, 18-21, and 23-26 were cancelled, and claims 33-37 were added on March 19, 2002. No claim has been amended.

Accordingly, claims 1-6, 13-15, and 33-37 are currently under examination. Claims 7, 16-17, 22, and 27-32 are withdrawn from consideration.

Applicant timely traversed the restriction of Group IV (claim 16) from the group under examination, and requested that claim 16 be rejoined into the application upon determination that the subject matter in the elected group is patentable, pursuant to MPEP § 821.04.

All claims under examination stand finally rejected in the Office Action dated January 30, 2003. Applicant filed a Response under 37 CFR § 1.116 on March 3, 2003, making no amendments to the claims. The Response has been entered into the file.

Summary of the Invention

The claimed invention provides ovine cells, tissues, and animals that have been engineered for reduction or elimination of the carbohydrate epitope Galα(1,3)Gal, which ovine cells normally express. This epitope is present, because it is a foreign antigen against which

humans have naturally occurring antibody. Elimination of Galα(1,3)Gal from the tissue helps render it suitable for use in human transplantation therapy.

The epitope is produced by the enzyme α(1,3)galactosyltransferase (EC 2.4.1.124), which adds galactose at the α(1,3) position to membrane-anchored Galβ(1,4)GlcNAc acceptor substance. Inactivation of the α(1,3)galactosyltransferase locus at both of the two alleles (a homozygous knockout) eliminates the enzyme from the cell, which in turn prevents the Galα(1,3)Gal epitope from being formed.

The invention is made possible by the discovery and isolation of the sheep α(1,3)galactosyltransferase (α1,3GT) gene and its genomic clone. The sequence of the gene is provided in the application (Example 1), and clones are deposited with the NCIMB in the United Kingdom in support of this application (page 51).

The specification illustrates how the α1,3GT sequences can be used to create targeting vectors (Example 3), which can then be used to inactivate the α1,3GT gene in isolated sheep fibroblasts from different strains (Examples 4 and 5). Heterozygous (single knockout) animals had been created from the targeted cells by the time the application was filed (Example 6), according to standard techniques in the field of animal cloning. Homozygous knockout animals can be made by using a double knockout cell in the cloning process, or by cross-breeding heterozygous knockout animals (pages 37-41). The cells and tissue made during the course of this work are then characterized (pages 41-43) to verify that they have the characteristics of the claimed invention — either at the genetic level (inactivation of the α1,3GT gene) or at the phenotypic level (reduced expression of the Galα(1,3)Gal epitope).

Issues

In the Office Action dated January 30, 2003 (Paper 17), the claims under examination were finally rejected under the both written description requirement and enablement requirement of 35 USC § 112 ¶ 1 — "for reasons of record advanced on Paper Nos: 7 and 12", and for additional reasons given in the Action. Still further reasons are given in the Advisory Action dated May 6, 2003. Applicant has diligently responded to each of the challenges raised — but because the challenges in the Office Actions are raised cumulatively, it is difficult to ascertain which issues have been resolved, and which issues remain a concern.

The central issue appears to be the fact that the specification does not contain a report of a homozygous α1,3GT knockout sheep actually having been made.

In responding to the Office Actions, applicant has pointed out that the specification provides the sheep α1,3GT sequence, α1,3GT targeting vectors, α1,3GT knockout cells, and heterozygous α1,3GT knockout fetal sheep. It further provides a full description of how homozygous knockout animals can be made using standard cloning techniques. Publications in the art show that once the α1,3GT sequence has been provided for a particular species, the making of homozygous α1,3GT knockouts for that species pose no particular difficulties.

Nevertheless, it is the Examiner's position that applicant is required to have a working example of an actual homozygous α1,3GT knockout sheep in order for the claims to be either described or enabled by the specification. The Examiner draws from MPEP § 2164.03 the proposition that "the physiological art in general is . . . unpredictable", and refers to published articles to support the contention that the making of transgenic animals is unpredictable.

This issue is the central focus of the argument that follows. Ancillary issues are addressed in the footnotes.

Grouping of claims

For purposes of this appeal, the claims are divided into the following groups. The claims do not stand or fall together, for reasons explained in the arguments that follow.

- Claims 1 and 2
- Claim 3
- Claims 4 and 33 to 37
- Claim 5
- Claim 6, 14, and 15
- Claim 13

ARGUMENT

Claims 1-6, 13-15, and 33-37 stand rejected under 35 USC § 112 ¶ 1 as not being adequately described or enabled by the specification.

The central issue appears to be the fact that the specification does not contain a report of a homozygous α1,3GT knockout sheep actually having been made.

Of course, the Board will recognize that there is no legal requirement that an actual working example be provided in the specification in order for a patent disclosure to be enabling.

> It is well established in the law that a specification can adequately describe the manner and process of making an embodiment of an invention, whether or not it has actually been conducted. Use of prophetic examples does not make a patent non-enabling. The burden is on the person challenging the patent to show . . . that the prophetic examples together with other parts of the specification are not enabling. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 224 USPQ 409 (Fed. Cir. 1984).

> Nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided though broad terminology or illustrative examples. *In re Wright*, 27 USPQ2d 1510 (Fed. Cir. 1993).

It is applicant's position that the claims are fully described and enabled, *inter alia* because the only elements missing from the working examples can be achieved as a matter of routine experimentation by the skilled reader.

Specifically, the description provides the newly discovered nucleotide sequence of the sheep α1,3GT cDNA (SEQ. ID NO:1) and the α1,3GT gene (SEQ. ID NOs:14 to 25). This is supported by a biological deposit (NCIMB Accession No. 41056) comprising cloned genomic DNA. Example 3 illustrates how the α1,3GT sequences can be used to create targeting vectors, which can then be used to inactivate the α1,3GT gene in isolated sheep fibroblasts from different strains (Examples 4 and 5). Heterozygous (single knockout) fetal sheep were created from the targeted cells by the time the application was filed (Example 6), according to standard techniques in the field of animal cloning.

Homozygous knockout animals can be made by using a double knockout cell in the cloning process, or by cross-breeding heterozygous knockout animals (pages 37-41). The cells and tissue made during the course of this work are then characterized (pages 41-43) to verify that they have the characteristics of the claimed invention — either at the genetic level (knockout of the α1,3GT gene) or at the phenotypic level (reduced expression of the Galα(1,3)Gal epitope).

Furthermore, the homozygous knockout cells of claims 4, 5, and 33-37 can be made without making a homozygous knockout animal, or doing any animal cloning at all (page 40, line 20 to page 41, line 13).

In requiring applicant to provide a working example of a homozygous α1,3GT knockout sheep, the Examiner has relied on the assertion that the area of making knockout animals is generally unpredictable. However, the Examiner has raised no specific technical issue why the making of a knockout sheep should pose special technical difficulties — difficulties that were not experienced in making α1,3GT knockouts in two other animal species.

Accordingly, the Office has not met its burden of establishing a *prima facie* case for non-patentability under 35 USC § 112 ¶ 1.

Nevertheless, in responding to the Office Actions, applicant has explained how it is possible to make a homozygous α1,3GT knockout sheep without undue experimentation, and why the phenotype is predictable.

1. Sheep cells having an inactivated α1,3GT allele can readily be produced.

One embodiment of the invention disclosed in this application are sheep cells in which the α1,3GT gene has been inactivated. This is claimed directly in claim 4 and claims 33-37. As explained in the specification, α1,3GT knockout cells can also serve as nuclear donors for the making of knockout animals by nuclear transfer (animal cloning).

The application newly provides the sheep α1,3GT gene, and describes and illustrates how to make targeting constructs for the purposes of creating α1,3GT knockout cells. Methods for using gene sequences to inactivate the corresponding gene in living cells are described extensively in the art.

- *Gene Knockout Protocols,* by Martin J. Tymms (Editor), Ismail Kola (Editor). 431 pages; Humana Press; 1st edition (January 15, 2001).
- *Laboratory Protocols for Conditional Gene Targeting,* by Raul M. Torres, Ralf Kuhn. Oxford University Press (October 1997).
- *Homologous Recombination and Gene Targeting,* by John Sedivy. MacMillan Pub Co. (November 1991).

- *Gene Targeting: A Practical Approach*, by Alexandra L. Joyner (Editor). Oxford University Press; ISBN: 019963792X; 2nd edition (February 15, 2000).
- *The Gene Knockout Factsbook* (2-Volume Set), by Tak W. Mak (Editor), Josef Penninger (Editor), John Roder (Editor), Janet Rossant (Editor), Mary Saunders. 1140 pages; Academic Press; 1st edition (November 15, 1998).
- *Gene Targeting Protocols (Methods in Molecular Biology, Vol 133)*, by Eric B. Kmiec (Editor), Dieter C. Gruenert (Editor). Humana Press (January 15, 2000).

Figures 9-15 in the application provide illustrations of targeting constructs based on the α1,3GT sequence that will inactivate the α1,3GT gene by removing exon sequences. Figure 16 illustrates the successful targeting and deletion of Exon 4, using the p0054 vector[1]. In Example 5, similar targeting was demonstrated using the same constructs on a different sheep strain cell line[2].

Thus, the skilled reader may use the constructs provided in the working examples, or design their own knockout strategy, by applying the sheep α1,3GT sequence using standard procedures in order to make the knockout cells of the invention.

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[1] The Office Action of May 21, 2002 (page 7) indicates concern that the specification provides no direct evidence for successful targeting of Exons 8 and 9. Nevertheless, targeting Exons 8 and 9 should be achievable without undue experimentation. In any event, it is not necessary to target Exons 8 and 9 to practice the claimed invention. It is only necessary to eliminate the translation start or remove enough of the gene to prevent the gene product from being functional. The targeting of Exon 4 (as effected in the working examples), or some other portion of the gene, or various portions in combination will be sufficient for the purpose of inactivating the α1,3GT gene in a sheep cell. The enablement requirement is met if the description enables *any mode* of making and using the claimed invention. *Engel Industries, Inc. v. Lockformer Co.*, 20 USPQ2d 1300 (Fed. Cir. 1991), emphasis added.

[2] The Office Action of November 23, 2001 (page 8) says that the specification is enabled for homozygous inactivation of the α1,3GT gene in the Finn Dorset strain of sheep, but not in other strains. It is recognized in the art of homologous recombination that a mismatch of about 1% (i.e., identity of about 99%) is well tolerated when using targeting constructs to cause gene inactivation. The difference between strains of the same mammalian species typically falls within this range. The α1,3GT sequence and targeting constructs used in the working examples were obtained from Black Welsh Mountain fibroblasts (Example 1). They have been used to successfully inactivate the α1,3GT gene in both Black Welsh Mountain sheep cells (Example 4), and Finn Dorset sheep cells (Example 5). This confirms that the claimed invention is enabled for different strains within the ovine species.

2. Sheep cells that are homozygously inactivated at the α1,3GT locus can readily be produced

Inactivating a single α1,3GT gene in a cell creates a heterozygous knockout. In order to prevent expression of the Galα(1,3)Gal epitope on the cell surface, both α1,3GT alleles must be inactivated (a homozygous knockout). As described in the specification, homozygous α1,3GT knockout cells according to claims 4 and 33-37 can be made using cultured cells, or by harvesting cells from a homozygous knockout animal.

An extensive description of how to make α1,3GT homozygous knockout cells in culture is provided in the specification beginning on page 40, line 20. Several techniques are explained, including sequential targeting of the two alleles by any one of these methods:

- Using a step-wise increase in antibiotic concentration to knock out both alleles (page 40, lines 24-27, citing U.S. Patent 5,589,369)
- Using two different antibiotics to sequentially knockout each allele (page 40, line 27 to page 41, line 4, referring to targeting constructs shown in Figures 9 and 11)
- Knocking out the first allele, and then retargeting and selecting homozygous knockout cells using an antibody that recognizes the Galα(1,3)Gal epitope (page 41, lines 8-13)

In the final Office Action of January 30, 2003, the claims were rejected under 35 USC § 112 ¶ 1 apparently because the Examiner did not believe that α1,3GT knockout sheep could be made. Applicant responded in part by pointing out that claims 4 and 33-37 *did not require the making of α1,3GT knockout sheep*, since homozygous knockout cells can be made in culture.

Subsequently, a new ground of rejection was stated in the Advisory Action of May 6, 2003. The Examiner refers back to publications directed towards animal cloning to support the

contention that homozygous α1,3GT knockout cells are difficult to make in culture[3]. On this basis, the Examiner indicates that the claims to knockout cells are not enabled[4]. Again, the Examiner is essentially requiring applicant to provide evidence of actual reduction to practice in order to meet the requirements of § 112 ¶ 1.

U.S. Patent 5,589,369 referred to in the specification describes and claims a system for making homozygous knockout cells[5]. Since this is an issued patent, 35 USC § 282 requires that the patent be presumed valid, and therefore enabled under 35 USC § 112 ¶ 1. The Examiner has not explained why the making of α1,3GT knockout cells would pose special difficulties that prevent the method of U.S. Patent 5,589,369 (or the other alternatives described in the specification) from being implemented as a matter of routine experimentation.

The steps referred to in Sections 1 and 2 are sufficient to enable the full scope of the cells in claims 4 and 33-37.

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[3] The Advisory Action refers to the article by Phelps et al., Science 299, 411-414, 2003. Heterozygous α1,3GT knockout pig cells were targeted a second time to obtain homozygous knockout cells, for the purpose of making homozygous knockout pigs. As it turns out, the second allele was inactivated not by homologous recombination, but by a fortuitous mutation event. Of course, this doesn't mean that homologous recombination doesn't' work; only that the single positive event ultimately cloned out of the system happened to have been achieved in an unexpected manner. The Advisory Action quotes col. 3 of page 413, which attributes the finding of the cell with the desired phenotype to a "powerful selection method". The method is described in col. 1 of page 412, and involves selecting targeted cells using a toxin that eliminates cells still expressing the Galα(1,3)Gal epitope. *An equivalent method is described in the specification on page 41, lines 9-11,* which teaches using an epitope-specific antibody to cause complement-mediated lysis of cells still expressing the Galα(1,3)Gal epitope. Thus, the patent application provides all the tools needed for the skilled reader to make a α1,3GT knockout cells in a manner that is equivalent to the Phelps method. As explained in Section 4 below, the Phelps article also confirms that this patent application enables the making of a α1,3GT knockout sheep.

[4] Of course, this is contrary to the position taken in the Office Action of November 23, 2001. On page 8, the Office Action says that the specification is enabled for homozygous inactivation of the α1,3GT gene in Finn Dorset sheep.

[5] US 5,589,369 (Seidman and Jakobovits, Cell Genesys) is entitled "Cells homozygous for disrupted target loci". Claim 1 covers "A method for making diploid mammalian cells homozygous for disrupted target loci . . . comprising . . . (a) introducing into diploid mammalian cells a construct . . . comprising a selectable marker gene . . . (b) growing the cells . . . in said selective medium at a first level of selective agent; (c) subjecting the population of cells . . . to a level of selective agent greater that said first level . . . and (d) isolating said cells . . ."

3. Animals having an inactivated α1,3GT allele can readily be made from α1,3GT inactivated donor cells by nuclear transfer

Some claims in the application involve or are facilitated by the making of a cloned animal from α1,3GT knockout cells. A central aspect of this invention relates to the discovery and characterization of the sheep α1,3GT gene, and its use for inactivating the α1,3GT gene inside cells. The α1,3GT knockout cells explained in Sections 1 and 2 above can be made into knockout animals using standard methods known in the art.

Several methods are available for making genetically modified animals from genetically altered cells. The specification explains extensively on pages 37-41 that animals can be cloned from a suitable donor cell by nuclear transfer. This is proven technology that created Dolly the sheep. The nuclear transfer method has been fully described and enabled in issued U.S. patents 6,147,276 and 6,252,133 (Campbell & Wilmut, Roslin Institute).

There is no reason to believe that genetically altering the donor cell would affect its suitability as a nuclear donor. To the contrary. A number of published experiments confirm that cloned animals may readily be made from genetically altered cells according to the Campbell & Wilmut method.

1. Uchida et al. (Transgenic Research 10:577, 2001) report the production of transgenic miniature pigs by pronuclear microinjection. The Huntington gene cloned from miniature pig, was linked to rat enolase promoter, and injected into pronucleus of fertilized eggs. Several of the offspring were determined to have the transgene by PCR and Southern analysis.
2. Bondoli et al. (Molec. Repro. Dev. 60:189, 2001) report cloned pigs generated from cultured skin fibroblasts derived from a boar with an H-transferase transgene. Two healthy piglets resulted from nuclear transfer by fusion of fibroblasts that had been extensively cultured with enucleated oocytes.
3. Lai et al. (Molec. Repro. Dev. 62:300, 2002) report a transgenic pig expressing green fluorescence protein. Fetal-derived fibroblast cells were transduced with the GFP gene, and then cloned into porcine oocytes. A healthy transgenic piglet was obtained that expressed GFP.

4. McCreath et al. (Nature 405:1004, 2000) report transgenic sheep made by nuclear transfer from fibroblast donors in which different transgenes were targeted into the alpha1(I) procollagen locus.
5. Lai et al. (Science 295:1089, 2002) report production of α(1,3)galactosyltransferase knockout pigs by nuclear transfer cloning. The pigs were produced by nuclear transfer, using clonal fetal fibroblast cell lines as nuclear donors.
6. Dai et al. (Nature Biotech 20:251, 2002) also report production of α(1,3)galactosyltransferase knockout pigs by nuclear transfer cloning. The pig α1,3GT gene was disrupted in both male and female porcine primary fetal fibroblasts, which were then used for nuclear transfer. Six clonal fetal piglets were obtained, of which five were normal weight and apparently healthy. Southern blot analysis confirmed that the five piglets contained one disrupted α1,3GT allele.
7. Denning et al. (Nat. Biotechnol 19:559, 2001) describe the deletion of the alpha(1,3)galactosyl transferase (GGTA1) gene and the prion protein (PrP) gene in sheep. Eight pregnancies were maintained to term and four PrP-/+ lambs were born.

These references confirm that both pigs and sheep can be cloned by the Campbell & Wilmut method using genetically altered cells to make genetically modified animals. References 5 to 7 are of particular interest, because they illustrate that heterozygous α1,3GT knockout animals can readily be made by nuclear transfer of heterozygous knockout cells. All the evidence of record indicates that the cloning of sheep according to the Campbell & Wilmut method is no more difficult if the cell used as the nuclear donor has been genetically altered.

4. Animals that are homozygous for inactivated α1,3GT can readily be produced

The skilled reader has at least three options by which to make a sheep in which both α1,3GT alleles have been inactivated:

1. Homozygous knockout cells can be made in culture as explained in Section 2 above. They are then used as nuclear donors to make homozygous knockout animals by the animal cloning method of Campbell & Wilmut (specification: page 37-41).
2. As an alternative, a nuclear donor cell with α1,3GT inactivated on one allele is used to produce a heterozygous knockout animal. Cells are harvested for a second round of targeting. This generates homozygous knockout cells, which can then be used to generate homozygous knockout animals by a second cloning event (specification: page 41, line 17).
3. Another alternative again involves making a heterozygous knockout animal to start. However, in this case, heterozygous knockout animals are simply cross-bred to produce a homozygous knockout animal (specification: page 41, line 14). This requires time for breeding the second generation, but in some ways is the most straight-forward option.

Since these technologies are all in wide-spread general use, the only relevant question in relation to the invention claimed in this application is *whether knocking out both α1,3GT alleles would somehow compromise the viability of the animal.*

In fact, we know this not to be the case. Humans and other Catarrhine primates are exceptions amongst mammalian species as not having an expressed α1,3GT gene. We seem to get along quite well without it. The α1,3GT gene has been obtained from two other species that normally express it, and used to create homozygous knockouts without difficulty.

Furthermore, the α1,3GT gene has successfully been knocked out in two mammalian species that normally express it.

- U.S. Patent 5,849,991 (Cols. 48-57) describes the isolation of the mouse α1,3GT gene, and then using it to make homozygous α1,3GT knockout mice.
- Phelps et al., Science 299, 411-414, 2003 describe production of homozygous α1,3GT knockout pigs using the pig α1,3GT gene[6].

The α1,3GT knockout mice of the '991 patent were made by targeting one α1,3GT locus in mouse embryos to make heterozygous knockout, and then cross-breeding to obtaining the homozygous knockouts (option 3, above). Such mice have been used extensively in labs around the world for immunological and transplant studies, and have the usual features of animals of the murine species — with the exception that they lack the Galα(1,3)Gal epitope on their cells.

The knockout pigs of Phelps et al. were also made according to the methods described in this patent application (option 2). First, the pig α1,3GT gene was used to make heterozygous knockout donor cells, which were then used to clone heterozygous knockout pig (page 412, col. 1; described in the present application *inter alia* on page 38, line 5 to page 40, line 19; and page 41, line 22 to page 42, line 5). Next, homozygous knockout cells were made by a targeting the other allele in the donor cells using a knockout vector, and selecting cells deficient in the Galα(1,3)Gal surface antigen (page 412, col. 1; described in the present application *inter alia* on page 41, lines 9-13 and 17-20; and page 42, lines 6-16). Finally, double knockout cells were used as donor cells for nuclear transfer to produce homozygous knockout animals (abstract; described in the present application *inter alia* on page 38, line 9 to page 40, line 19).

Four double-targeted female piglets were produced by Phelps et al., of which three had α1,3GT inactivated on both alleles (page 412, col. 3 ff).

Based on the precedents of humans, other Catarrhine primates, and homozygous knockout mice, and pigs, there is no reason to believe that homozygous knockout sheep would not be equally viable, and equally straight-forward to produce by any one of the three approaches described in the specification.

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[6] The pig α1,3GT gene sequence had already been disclosed in U.S. Patent 5,821,117, which includes claims both to the gene sequence and to cells having an inactivated α1,3GT gene.

5. Cells from homozygous knockout animals will have cells and tissues lacking the Galα(1,3)Gal xenoantigen

As described in the specification, the α1,3GT gene is uniquely responsible for forming the Galα(1,3)Gal xenoantigen in non-Catarrhine mammals. An animal that is homozygous for inactivation of the α1,3GT gene would therefore lack the enzyme responsible for making the Galα(1,3)Gal epitope, and would necessarily be deficient in expressing the epitope at the cell surface.

The homozygous knockout mice in U.S. Patent 5,849,991 confirm this expectation. Peripheral blood monocytes and splenocytes from the homozygous knockouts were analyzed for presence of the Galα(1,3)Gal antigen using the IB4 lectin, in a manner comparable to what is described in the specification of the present application on pages 41-43. Wild-type mice showed high degree of staining, while knockout mice showed minimal staining, confirming that the tissue was devoid of the Galα(1,3)Gal antigen (U.S. 5,849,991, Cols. 48-52). As expected, since Galα(1,3)Gal is not a self-antigen in these mice, they form naturally occurring antibody against it, as do humans (Chong et al., Transpl Immunol 8:129-37, 2000).

Similarly, the homozygous knockout pigs in the article by Phelps et al. This is shown in are devoid of antibody-detectable Galα(1,3)Gal. See Fig. 1., clones B1-1, B1-2, and B1-4 (the three correctly targeted clones), and Fig. 2.

In summary, techniques suitable for preparing α1,3GT knockout animals are generally known in the art, and referenced in the specification. It has not previously been possible to make α1,3GT knockout sheep, simply because the sheep α1,3GT gene was not previously available. Now that the sheep α1,3GT gene has been discovered and characterized, it is now straight forward to produce sheep tissue which is devoid of Galα(1,3)Gal, or which has been inactivated for the α1,3GT gene on one or both alleles, using techniques already proven to be effective in the mouse and the pig.

Predictability of the Art

In requiring applicant to provide full reduction to practice of a homozygous α1,3GT knockout sheep, the Examiner asserts that the "physiological art" in general, and the art of making genetically altered animals in particular, is unpredictable. In support of this assertion, two publications have been put forward in the Office Actions.

The Office Action of November 23, 2001, refers to an article by Linder et al. (Lab. Anim. NY 30:34, 2001) as indicating that the resulting phenotype of a targeted gene mutation would vary among different strains of animals because of the collective effect of different genes in the host. The issue raised in the article is that inactivation of a gene may generate different phenotypes in particular inbred strains of mice. This is because the genes exemplified do not directly generate the phenotype being measured, but cause the phenotype to change by complex interaction with other gene products.

In contrast, the α1,3GT gene targeted in the present invention is directly responsible for generating the enzyme that builds the Galα(1,3)Gal epitope[7]. Accordingly, none of the concerns raised in the Linger article are relevant. The homozygous α1,3GT knockout mice described in U.S. Patent 5,849,991 were made by breading heterozygous knockout mice, and the line has continued to bred true. All evidence indicates that α1,3GT knock-out animals reliably breed towards absence of the Galα(1,3)Gal epitope, as expected.

The Advisory Action of May 6, 2003, refers to the article by Denning et al. (coinventors on this patent application) entitled *Deletion of the α(1,3)galactosyl transferase (GGTA1) gene and the prion protein (PrP) gene in the sheep* (Nature Biotechnol. 19:559, 2001). The Examiner is apparently concerned that the first α1,3GT knockout sheep clones died *in utero*. But the

[7] Table 2 in the Linder article shows that knocking out the gene for IL-2 can cause splenomegaly, inflammatory bowel disease, or generalized autoimmune disease, depending on the genetic background. A similar observation is made for *ob/ob* obese mice, which have a homozygous mutation in the leptin gene. The target genes in these studies are both endocrine molecules (IL-2 and leptin) which mediate a complex response pathway between different cells. In contrast, the present invention is directed at inactivating a gene that puts a terminal sugar residue onto the carbohydrate substrate N-acetyl lactosamine, which all ovine animals express. Accordingly, no inter-strain variation is expected. Another issue raised in the Linder article is that a cross-over event that occurs during breeding may separate a mutant gene from the phenotype being used to follow the breeding, if the phenotype is not directly encoded by the mutated gene. This is not a concern for the present invention, because the presence of an inactivated target gene can be detected directly — either by PCR analysis (Figures 16 and 17), or by detection of the Galα(1,3)Gal epitope on the animal's cells.

Denning article was only a preliminary report. It does not support the contention that gene targeting in sheep is an uncertain process.

On the contrary — the article provides several illustrations of the viability of the claimed invention:

- Sheep cells can be correctly targeted for inactivation of the α1,3GT gene. See Figure 2, panel (A); and Table 1. This provides a direct illustration of the making of the knockout cells covered by claims 4 and 33-37.
- Targeted cells can be used for nuclear transfer. There are three examples: a) the α1,3GT knockout cell gave rise to viable embryos; b) the PRP knockout cells gave rise to 3 live births; c) viable animals have been produced that were successfully targeted at the COL1A1 locus (ref. 5, discussed on page 559, col. 1).
- *Knocking out the α1,3GT gene does not decrease viability of the embryo.* See the data in Table 2. Nuclear transfer with untransfected donor cells (7G65F4) gave rise to 5 viable fetuses at day 60 in 33 attempts (a 13% success rate). Nuclear transfer of cells treated with the α1,3GT vector but not inactivated (4H2) gave rise to 2 viable fetuses in 23 attempts (an 8% success rate). Nuclear transfer of cells containing an inactivated α1,3GT gene (3C6 and 5E1) gave rise to 5 viable fetuses in 21 attempts (a 19% success rate). Ergo, the success rate for cloning sheep by nuclear transfer is not further reduced by knocking out the α1,3GT gene. If anything, there was actually an improvement in cloning frequency using the correctly targeted cells.

Thus, the Denning article confirms that the generation of α1,3G knockout sheep *poses no undue difficulty* beyond what is usually entailed in producing cloned knockout mammals by nuclear transfer. The method has been used successfully for recombination at the COL1A1 locus in sheep (McCreath et al., Nature 405:1066, 2000), and the α1,3GT locus in pigs (Phelps et al., *supra*).

The sheep α1,3GT knockout project reported in the Denning article lost its funding, and it is for this reason that knockout sheep were not ultimately produced by these authors. There is no question that large animal cloning is a costly and time-consuming process, whether or not *the nuclear donor cell has any kind of genetic alteration.* But that does not mean that the claimed invention is in any way inadequately described or enabled. The cloning step required to complete the invention claimed in this application, while costly, is entirely straight forward. *It can be*

accomplished without undue experimentation, well within the Wands standard[8]. There is nothing missing from the specification that the skilled reader needs in order to put this invention into practice[9].

Applicant should not be penalized for disclosing their invention before they completed actual reduction to practice. To the contrary: an objective of the patent law is to disseminate advances in the art and thereby provide a public benefit as soon as possible. The inventors of the claimed invention fully entered into the spirit of the public policy objective by filing their patent disclosure soon after critical elements of the invention had been made (isolation and characterization of the sheep α1,3GT gene). They knew full well that this places the invention in the hands of the public through the implementation of standard cloning technology.

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[8] *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988). In *Wands*, the patent application claimed monoclonal antibodies of a particular specificity and affinity. The PTO contended that only 2.8% of the hybridomas obtained were proven to fall within the claim, and thus the claim was not enabled. The Court held that Wands was fully enabled, because it was standard practice to screen negative hybridomas in order to find one that makes the desired antibody.

[9] Except, of course, funding. But 35 USC § 112 ¶ 1 only requires the applicant to provide the skilled reader with the knowledge required to make and use the invention — not the financial resources that may be needed to complete the project.

Patentability of claim groups

- Claims 6, 14, and 15 cover an ovine animal that is homozygous for inactivation of the α1,3GT gene. It meets the description and enablement requirements of 35 USC § 112 ¶ 1 for reasons already explained. Briefly, the specification provides the sheep α1,3GT gene, α1,3GT targeting constructs, and α1,3GT knockout sheep cells. α1,3GT knockout sheep can be made by applying standard animal production technology referred to in the specification.
- Claims 4 and 33 to 37 have different requirements under 35 USC § 112 ¶ 1. Heterozygous and homozygous cells can be made in culture without producing a knockout animal.
- Claim 5 depends from claim 4, and requires only that the process for making the cell involve at least one nuclear transfer event. This may involve the making of a cloned animal, or just the making of a single cell or cell culture by nuclear transfer.
- Claim 13 depends from claim 4, and covers the use of heterozygous or homozygous α1,3GT knockout cells for making homozygous α1,3GT knockout sheep.
- Claim 3 covers a cell or tissue that does not express α1,3GT. It can be harvested from a α1,3GT knockout animal, or (depending on the tissue), it can be produced in culture.
- Claims 1 and 2 cover tissue devoid of the Galα(1,3)Gal epitope, made by any suitable method. These claims do not explicitly require that the α1,3GT gene be inactivated on either or both alleles.

Summary

The Office has not established a prima facie case for lack of description or enablement for the claimed invention. Heterozygous knockout cells and sheep have been made using the α1,3GT targeting vectors described in the specification. There is no basis to believe that making a homozygous knockout will compromise the viability of the animal in any way. In fact, there is abundant evidence from other species that the making of such animals is straight forward. And the Galα(1,3)Gal epitope cannot be formed if the enzyme that forms it has been deleted.

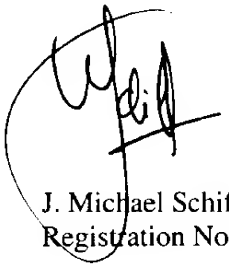
Applicant should not be required to provide complete reduction to practice in order to demonstrate description and enablement, since this is not the legal standard. The only elements missing from the working examples can be implemented by those skilled in the art without undue experimentation. A patent need not teach, and preferably omits, what is well known in the art. *In re Buchner,* 18 USPQ2d 1331, 1332 (Fed. Cir. 1991).

Accordingly, the claimed invention is fully described and enabled in the specification, thereby complying with the patentability requirements of 35 USC § 112 ¶ 1.

Applicant respectfully requests that rejection of all claims under examination be reversed.

Should the Patent Office determine that an extension of time or any other relief is required for further consideration of this application, applicant hereby petitions for such relief, and authorizes the Commissioner to charge the cost of such petitions and other fees due in connection with the filing of these papers to Deposit Account No. 07-1139, referencing the docket number indicated above.

Respectfully submitted,



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APPENDIX

Claims under Examination:

1. Ovine tissue devoid of antibody-detectable Galα(1,3)Gal determinants.

2. The tissue of claim 1, which is selected from the group consisting of lung tissue, kidney tissue, liver tissue, cardiac tissue, pancreatic tissue, and ocular tissue.

3. Isolated ovine cell or tissue that expresses glycosyl transferase enzymes but does not detectably express α(1,3)galactosyltransferase (α1,3GT).

4. An ovine cell which is heterozygous or homozygous for inactivation of an α1,3GT gene.

5. The cell of claim 4, produced by transfer of a nucleus from a donor cell heterozygous or homozygous for inactivation of an α1,3GT gene, to an enucleated recipient cell.

6. An ovine animal that is homozygous for inactivation of an α1,3GT gene.

13. A method for producing an ovine that is homozygous for inactivation of an α1,3GT gene, comprising providing an ovine embryo of cells according to claim 4, engrafting the embryo into the uterus of a female, birthing an ovine with an inactivated α1,3GT gene from the engrafted female, and if the birthed ovine has the α1,3GT gene inactivated on only one allele, then mating it with another ovine with an inactivated α1,3GT gene, thereby producing an ovine that is homozygous for inactivation of the α1,3GT gene.

14. A method for producing an isolated ovine cell that expresses glycosyl transferase enzymes but does not detectably express α1,3GT, comprising isolating the cell from an ovine homozygous for inactivation of an α1,3GT gene according to claim 6.

15. A method for producing ovine tissue devoid of antibody-detectable Galα(1,3)Gal determinants, comprising harvesting the tissue from an ovine homozygous for inactivation of an α1,3GT gene according to claim 6.

33. The cell of claim 4, which is a fibroblast.

34. The cell of claim 4, which is a kidney cell.

35. The cell of claim 4, which is a hepatocyte.

36. The cell of claim 4, which is a cardiac cell.

37. The cell of claim 4, which is an islet cell.

Claim Withdrawn from Examination for which Rejoinder has been Requested:

16. A method of xenotransplantation, comprising transplanting tissue devoid of antibody-detectable Galα(1,3)Gal determinants according to claim 1 into a mammal having circulating antibody against Galα(1,3)Gal determinants.

Other Claims Withdrawn from Examination:

7. A polynucleotide construct effective for inactivating an α1,3GT gene in an ovine cell.

17. An isolated polynucleotide that comprises a sequence of at least 30 consecutive nucleotides with at least one of the following properties:

a) it is contained in SEQ. ID NO:1 or any of SEQ. ID NOs:14 to 25, but not in any of SEQ. ID NOs: 3, 5, 7, 9, 11, and 13;

b) it is contained in phage **B**, **C** and **G** deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061; but not in λ-phage or any of SEQ. ID NOs: 3, 5, 7, 9, 11, and 13; or

c) it hybridizes under stringent conditions to a polynucleotide with the sequence in SEQ. ID NO:1 or any of SEQ. ID NOs:14 to 25, but not to a polynucleotide with the sequence in any of SEQ. ID NOs: 3, 5, 7, 9, 11, and 13

22. An isolated polypeptide that comprises a sequence of at least 10 consecutive amino acids with at least one of the following properties:

a) it is contained in SEQ. ID NO:2 but not in any of SEQ. ID NOs: 4, 6, 8, 10, and 12;

b) it is encoded in phage **B**, **C** and **G** deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061., but not encoded in λ-phage or present in any of SEQ. ID NOs: 4, 6, 8, 10, and 12; or

c) it is at least 80% identical to 15 consecutive amino acids contained in SEQ. ID NO:2, wherein said sequence is not present in any of SEQ. ID NOs: 4, 6, 8, 10, and 12

27. An isolated polynucleotide comprising a sequence encoding a polypeptide according to claim 22.

28. An isolated polyclonal antibody or a monoclonal antibody that binds specifically to a polypeptide with the sequence SEQ. ID NO:2 but not to a peptide with the sequence present in any of SEQ. ID NOs: 4, 6, 8, or 10.

29. An assay method for determining α1,3GT expression by a cell, comprising contacting a polynucleotide according to claim 17 with the cell or with mRNA or cDNA obtained from the cell, detecting any hybrids that form as a result, and correlating presence of the hybrids with expression of α1,3GT by the cell.

30. A method for producing the antibody specific for sheep α1,3GT, comprising immunizing an animal or contacting an immunocompetent particle with a polypeptide according to claim 22.

31. A method for preparing a Galα(1,3)Gal determinant, comprising contacting a galactose acceptor saccharide with the polypeptide of claim 26 in the presence of UDP-galactose.

32. An assay method for determining α1,3GT in a sample, comprising preparing a reaction mixture comprising the sample and an antibody according to claim 28 under conditions that permit the antibody to complex with α1,3GT, and correlating any complex formed with the presence or amount of α1,3GT in the sample.

ENCLOSURES

- U.S. Patent 5,849,991. *Mice homozygous for an inactivated .alpha. 1,3-galactosyl transferase gene.* (Specification, page 3, line 10)

- U.S. Patent 5,821,117 *Xenotransplantation therapies.* (Specification, page 3, line 9)

- Denning et al., Nature Biotechnology 19:559, 2001. *Deletion of the alpha(1,3) galactosyl transferase (GGTA1) gene and the prion protein (PrP) gene in sheep.* (IDS of October 22, 2001)

- Phelps et al., Science 299, 411-414, 2003. *Production of α1,3-galactosyltransferase deficient pigs.* (IDS of March 31, 2003)